Social factors and apolipoprotein E and angiotensin genotypes are associated with allostatic load among American Samoans

Research Thesis

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ABSTRACT

Allostasis represents biological responses to stressors across physiological systems. Allostatic load (AL) accumulates as we successfully and unsuccessfully respond to morphological, social, and environmental stressors. AL appears to be amplified by senescent processes, predisposing individuals to chronic non-communicable diseases and predicting future morbidity and mortality. Individual responses to stressors are influenced by genes, social, and environmental factors. Similarly, age, sex, and education commonly associate significantly with AL across samples. We examined genotypes at apolipoprotein E and H, angiotensin converting enzyme (ACE), and atrial natriuretic peptide (ANP) loci and social factors in a sample of 273 American Samoans for associations with AL. Our main estimate of AL is based on seven secondary mediators of allostasis. We augment this with two additional models of AL, one including four additional aspects of body habitus and another including three aspects of glucose and lipid metabolism. We then determined if age, sex, or education and additional social factors influence associations of genotypes with AL. The apolipoprotein (apo) E 3*2 and ACE I*I genotypes were significantly associated with AL ($p<.05$). Neither apo H nor ANP genotypes were significantly related to AL, but sex and education were. Women showed significantly higher overall AL than men. Younger individuals (<55 years old) had higher AL than those 55 and over. Associations with education and social factors varied depending on how AL was constructed. Genotypes apparently modulate physiological dysfunction in American Samoans, likely altering risks for morbidity and mortality. However, these effects are modulated by age, sex, and social factors. Determining gene-environment-sociocultural interactions will aid understanding of how stressors lead to physiological dysregulation as assessed by AL.

BACKGROUND

Among the goals of human biological research is determining genetic influences on health, physical dysfunction, reproduction, and survival (Damon 1975; Crews 1994; Johnson and Wolinsky, 1994; Crews 2003; Crews et al., 2004; Smith et al., 2009; Brody et al., 2013). As anthropologists we examine relationships among stressors and stress responses across environmental and cultural settings to determine how these structure phenotypic variation (Baker 1984). Previously, we reported associations of apos E and H, ACE, and ANP with phenotypic variation among 273 residents of American Samoa (Crews et al., 1993; Crews and Harper, 1998, Crews et al., 2004). Here we extend these analyses to examine associations of genotypes at the same loci with allostatic load (AL). Our purpose is to further explore recently reported associations of ACE and other genotypes with AL (Smith et al., 2008; Brody et al., 2012).

Social (e.g., socio-economic status [SES], globalization, poverty), environmental (e.g., solar radiation, food, available nutrients), and somatic (e.g., internal injuries, vascular deficiencies, cellular senescence and loss) stressors alter homeostasis, producing stress responses (Goodman et al., 1992; Bindon et al., 1997; Flinn and England 1997; Goodman and Leatherman 1999; Decker 2000; Ellison 2001; McDade 2001; Crews 2003;). Stress responses to these and multiple additional stressors are measurable as biological, physiological, and functional alterations in somatotypes, biological age, hormones, and inflammatory markers (Sheldon et al., 1954; Katz et al., 1963; Damon 1975; Borkan et al., 1982; Shock 1985; Borkan 1986; Smith et al., 2008; Power and Schulkin, 2012; Theall et al., 2012). Here, we assess allostatic load (Table 1; see Seeman et al., 1997) to determine influences of genetic polymorphisms (apos E and H, ACE, and ANP) on physiological dysregulation. We then examine these associations while accounting for age, sex, and education in a sample of 273 American Samoans.

INSERT TABLE 1 HERE

Homeostasis and Allostatic Load

Homeostasis reflects the body's ideal state of constant physiological set points, while allostasis represents internal regulation of bodily functions to maintain a constant state (Berntson and Cacioppo, 2000; Schulkin 2004; Danese and McEwen, 2012), the primary goal of physiological response (Power and Schulkin 2012). Organisms generally maintain homeostasis via negative feedback to the brain, particularly the amygdala, hippocampus, and hypothalamus. Physiologically, stress response minimizes change amplitude, providing a self-regulating system responsive to perturbations secondary to stressors. Neurological responses maintain set points across multiple internal systems consistent with adequate function. Numerous aspects of physiology (e.g., blood oxygen, pH) must remain within specific ranges for survival; allostatic processes are what maintain function within homeostatically optimal ranges (McEwen and Gianaros, 2010). Allostatic processes promote survival and health by minimizing damaging effects of current stressors on one's body. Bodily systems, including the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system, support somatic homeostasis (McEwen 1998; Power and Schulkin, 2012; Theall et al., 2012). Stably regulating stressors and internal stress response is critical for survival and promotes longevity (Peters and McEwen, 2012). Cognitive adaptability and disruptions to processing also modulate individual responses to life's stressors.

Distinct from homeostasis, allostasis represents changes in physiological states and somatic responses to internal and external stressors designed to maintain homeostasis. Allostasis is continual, constantly altering physiology in response to prevailing environment, moving

somatic systems closer to homeostasis. Allostasic responses allow one's body to adapt constantly to ever-changing environments (Sterling and Eyer, 1988; Seeman et al., 1997; Schulkin 2004; Crews 2012, 2013). Multiple interacting systems maintain homeostasis as the soma responds to stressors and to additional alterations generated by ongoing allostasis. Individuals do not show identical stress responses when their homeostasis is threatened. Perceptions, previous experiences, and familiarity with a stressor influence strength and amplitude of stress response. Thus, given the same stressor, one person may activate greater stress response than another, leading to differences in their allostatic burdens and their ultimate physiological dysregulation (McEwen 1998). Continual allostatic responses or their failure may, over time, produce an allostatic load as allostatic responses themselves, or their loss, damage the soma. Across samples, including Japan, American Samoa, and the United States (USA), higher AL is associated with future risks for disease, cognitive declines, decreased immune function, frailty, and mortality (see review by Leahy and Crews, 2012; see also Schulkin 2004, Crews 2007, 2012).

An allostatic load (AL) results from both failed and adequate morphological and physiological stress responses (McEwen and Stellar 1993; McEwen 1998, 1999, 2000; Danese and McEwen, 2012). Multiple aspects of genes, environment, and culture, along with their interactions, influence adaptive and maladaptive responses to stressors (Baker, 1984; Sterling and Eyer 1988; Seeman et al 1997, 2001). Primary physiological modulators of stress response are glucocorticoids (mainly cortisol), catecholamines (adrenaline and noradrenaline), and serum dehydroepiandroesterone-sulfate (DHEA-S), a cortisol antagonist (Table 1). Secondary physiological and somatic responses to dysfunction of primary mediators include altered lipid metabolism as expressed by elevated triglycerides and total cholesterol, and low serum HDL-

cholesterol; poor glucose metabolism expressed as elevated fasting glucose/insulin and glycated hemoglobin; cardiovascular over-reactivity indexed by elevated blood pressure; and excessive energy storage as shown by a higher BMI and waist/hip ratio as well as larger skinfolds.

Joint assessment of these 10 biomarkers (Table 1) provides a useful measure of current AL within individuals (Seeman et al, 1997; McEwen et al, 1999; Power and Schulkin, 2012; Theall et al, 2012). Each biomarker either participates directly in allostatic response or is partly attributable to long-term exposures to stressors and allostatic hormones. Senescent processes also enhance one's AL, predisposing individuals to cardiovascular diseases, diabetes and earlier mortality (Crews et al, 2007). Essentially, one's AL represents one's decline from an "optimal" state of physiological function to an increasingly dysfunctional physiological phenotype. Across samples from different populations, age and sex are associated significantly with AL (see Leahy and Crews, 2012). Among those predisposed to higher AL in the USA are men, older individuals, those with low daily physical activity, incomes below the poverty line, or living in rural neighborhoods (Power and Schulkin, 2012; Theall et al., 2012). However, in some settings, for example, Japan, women show higher AL (Crews et al., 2012). Disparities exist between AL in European-American and African-American men after controlling for socioeconomic status and health behaviors, suggesting possible genetic or cultural influences (Duru et al., 2012). Genetic variation seems to predispose children towards developing higher AL (Caspi et al., 2010; Ganzel et al., 2010). Early developmental processes and biology also may influence AL risk and health disparities in later life (Geronimus et al., 2006).Multiple non-biological factors, for example, poverty, childhood stress and abuse, crime and alcohol use, also affect health and predict higher AL (Theall et al., 2012).

Health and social constructs also affect gene expression (Kim et al, 2012). Racism and negativity both contribute to higher AL (Duru et al, 2012). Additional behavioral factors, daily alcohol and caloric intake, are associated with dyslipidemia and dysglycemia, although such relationships vary by sex (Hishida et al, 2012), likely contributing to one's AL. In Sweden and American Samoa, job demands are positively associated with blood pressure and C-reactive protein (CRP) respectively, further linking both to AL (Bindon et al., 1997; Schnorpfeil et al, 2003; Hansson et al., 2008). Genes and lifestyle interact to produce phenotypic responses to chronic stressors. For example, Czerwinski et al (2004) reported smoking altered associations of LDL-cholesterol and triglycerides with genotypes.

In the USA, AL assessed using 10 biomarkers suggested by Seeman et al. (1997) is associated significantly with physical and cognitive declines and risks for non-communicable diseases that accompany aging. Across populations, local variation in AL likely is linked to genetic variance, lifestyles, and sociocultural characteristics. For example, children exposed to many stressors may be predisposed to higher AL and cumulative risk for cardiovascular disease. This may be related to maternal responsiveness during childhood (Evans et al., 2007). Among American Samoans, higher AL was observed in women than men and AL was significantly related to diabetes in both sexes, even when no biomarker of glucose metabolism was included in assessing AL (Crews et al., 2007). Previously, we examined associations of several candidate genes with body habitus, blood pressure and lipids among American Samoans (N=273: Bindon et al., 1997; Crews et al., 2004). Here, we examine associations of AL with genotypes, age, sex, and education in the same sample.

Previous Research

Apolipoproteins maintain structure and solubility of lipids, serve as ligands for cell-membrane receptors, and participate in lipid metabolism as coenzymes (Hegele and Breslow, 1987; Breslow, 1988). Previously, Crews and colleagues (1993) reported on six apolipoprotein loci in the sample used here: A-I, A-II, A-IV, C-II, E, and H. Alleles and genotypes at these loci show significant, but variable, associations with lipid levels across studies. Allele frequencies also vary across geographic regions and populations. For example, samples from Mexico have a significantly higher frequency of the APO E*3 alleles than do their European-American counterparts (Medina-Urrutia et al., 2004). Similarly, samples of Native Americans, including the Yanomami, have high frequencies of the APO E*3 allele and a dearth of the APO E*4 allele (Crews et al., 1993).

Angiotensin-converting enzyme (ACE) assists in blood pressure regulation (Crews and Harper, 1998). The ACE insertion-deletion polymorphism, specifically the I-I genotype is associated with greater risk of cardiovascular disease and higher mortality in "Caucasian" samples (see for example Morris et al., 1994). Additionally, the I-I genotype is linked to myocardial infarction (Cambien et al, 1992) and hypertriglyceridemia in Chinese samples (Hu et al, 2007). This genotype predisposes towards abnormal serum ACE concentration (Rigat et al, 1990). ACE also coregulates CRP, IL-6, and cortisol (Smith et al, 2009). Because AL predicts risk of degenerative disease and senescence, examining the ACE I-D polymorphism and APOs E and H separately may reveal genomic impacts on AL.

Atrial natriuretic peptide (ANP) variants also show significant associations with blood pressure. A 1989 study by Crews and Harper (published in 1998) demonstrated that American

Samoan individuals carrying the ANP top-top (TT) restriction site genotype had higher rates of diastolic hypertension (p=0.098) and elevated systolic blood pressure (p=0.157), although not significantly so. Additional research indicates associations of the ANP TT genotype with essential hypertension, cardiovascular diseases, and intermediate phenotypes in a Chinese sample (Hu et al, 2007).

Among American Samoans, apolipoprotein alleles and genotypes are related significantly to body habitus, lipids and glucose metabolism (Crews et al, 1993). In other samples, apolipoprotein alleles predict increased risks for hyperlipidemia, Alzheimer's disease, and coronary heart disease (Benderly et al, 2009). Both AL and allelic variability likely modulate risks for chronic diseases within populations, although specific associations probably vary (Medina-Urrutia et al, 2004; Enkhmaa et al, 2010). Here we determine how genotypic variation at two apolipoprotein loci, APO E and H, along with ACE and ANP, are associated with AL in a sample of 273 residents of American Samoa. In addition, we explore how age, sex, and education are associated with and may confound associations of genotypes with AL.

MATERIALS AND METHODS

During the 1970s-1990s, samples of Samoans residing in American Samoa, Western Samoa, Hawaii, and California, participated in a long-term study of human adaptability and changing environments, the Samoan Studies Project (SSP) (Baker et al., 1986). Populations of the South Pacific have been of particular interest due to their rapid increase in obesity and prevalence of morbidity and mortality from chronic diseases over the latter half of the 20th century (Bindon and Baker, 1985). One suggestion is genomic characteristics that previously

benefited fitness on these small islands may now predispose Samoans to obesity and associated health problems, including diabetes and cardiovascular diseases. In the 1980s, average BMI among American Samoan women was 33kg/m^2 and they averaged 1.60m in height, while American Samoan men averaged 30kg/m^2 and stood 1.80m high (Crews et al., 1993). BMI and multiple other anthropomorphic measures, for example, triceps skinfolds, are at higher mean levels among American Samoans than observed for most other populations, including the USA (Crews et al., 1991). However, mean total- and HDL-cholesterol are generally lower in Samoans than in U.S. samples (Pellitier & Hornick, 1986).

Among American Samoans, genotypic variation at the ACE, ANP, and apolipoprotein loci is related to body habitus and physiology. The most common apolipoprotein E allele, APO E*3, is significantly associated with higher body weight, percent trunk fat, subscapular/triceps skinfold ratio, and larger subscapular, suprailiac, and medial calf skinfolds (Crews et al, 1993). The APO E*4 allele also associates significantly with higher body weight, BMI, upper arm circumference, arm muscle circumference, and smaller suprailiac skinfold, along with lower blood pressure and higher pulse rate (Crews et al, 1993). No significant associations of APO H alleles with body habitus or physiology were observed in earlier studies. Elsewhere (Europe, Japan, and in Hispanic samples) no APO E allele showed a higher frequency among obese compared to normal weight participants (Crews et al, 1993).

Participants

During 1992, Crews and colleagues (1993; Crews et al 2004) examined apolipoprotein E, A-IV, and H loci, the ACE I-D polymorphism, and an ANP polymorphism in 273 (123 male, 150 female) residents of American Samoa. Participants were recruited within households

representing all four public health districts. Participating ‘Aigas’ (extended family households) were identified from the original 1976 cohort for the SSP. Those having members aged 55 and over at the time of the 1992 follow-up survey were contacted. The final sample consisted of household heads, their spouses, and several non-related older adults living within the study household at time of data collection (Bindon and Crews, 1993; Bindon et al., 1997; Crews et al., 2004, 2007).

Methods

Detailed methods of data acquisition are provided by Crews et al. (1992, 1993, 2004, 2007). All blood samples were in good condition for apolipoprotein, ACE, and ANP typing (Crews et al, 2004). Allelic and phenotypic frequency data for APO E and H also are described elsewhere (Crews et al, 1991b). Protocols for IEF-immunoblotting APO H and E are detailed elsewhere (Kamboh and Ferrell, 1987; Kamboh et al, 1987, 1988a,b; Sepehrnia et al, 1988b). Finger-sticks and a GLUCOSCAN® Blood Glucose Meter and GLUCOSCAN® Test Strips were used to determine fasting and post-load capillary blood glucose levels. The GLUCOSCAN® products were donated by Lifescan, Inc. Resulting values were multiplied by 1.15 to obtain their plasma equivalents (see Fitton and Crews, 1994; Crews et al., 2004).

The 273 study participants were examined for six dimensions and five indices of body habitus. Measured were weight, triceps, subscapular, suprailiac, and medial calf skin folds, and upper arm circumference. BMI (body mass index = weight(kg)/height(m^2)), percent trunk fat (ratio of subscapular to medial calf skinfolds), RFPI (relative fat pattern index: a ratio of subscapular skinfold to subscapular plus suprailiac skinfolds), and estimated arm muscle circumference (mid-upper arm circumference – (π x triceps skinfold/10)), measures of relative

weight, were calculated. Participants with skinfolds larger than 60 mm were assigned a value of 61 mm. Also recorded were self-reported sex and age (verified with official documents when available). Additional assessments included systolic and diastolic blood pressure, fasting and post-load glucose, total-cholesterol, triglycerides, glycated hemoglobin, HDL-cholesterol, LDL-cholesterol, and fasting and post-load insulin (Crews 2007). Techniques detailed by Weiner and Lourie (1969) were used for anthropomorphic measurements throughout the SSP. All methods are available in other publications (McGarvey and Baker, 1979; Bindon, 1984a,b; Bindon and Baker, 1985; Crews, 1985, 1988).

Participants were also administered a survey and questionnaire containing questions related to income, education level, smoking and drinking habits, physical activity levels, and personal and family history of chronic disease. These questions were used to construct a physical activity/drinking/smoking/blood pressure index. Associations of AL with all additional social variables were then analyzed.

Construction of AL

As suggested by Seeman et al (1997) and McEwen (2000), a score of 1 is assigned when a participant's value on any one biomarker (Table 1) falls in the quartile associated with the highest risk in population studies and zero when in any of the three other quartiles. Quartiles are determined from the observed sample distribution. These "scores" are added together. Thus, AL ranges from zero (no measures in the highest risk quartile) to the number of variables measured (all in the highest risk quartile). For every biomarker assessed here, except HDL-cholesterol, highest risk occurs in the upper fourth of the sample distribution. For HDL-cholesterol, a 1 was assigned for values in the lowest fourth of the distribution, the highest risk quartile. Here, three

different assays of AL are examined (Table 2). Model 1, the basic model, includes fasting insulin as a hormonal mediator of stress and all 6 secondary mediators proposed by McEwen and Stellar (1993) and Seeman et al (1997). AL assay 2 is a body habitus model. It augments Model 1 by including triceps and subscapular skinfolds, BMI, and RFPI. Model 3 is a metabolic model, augmenting Model 1 by adding LDL-cholesterol, triglycerides, and fasting glucose to AL. Previously, these estimates of AL were examined for variation by sex, age and Type II diabetes; significant differences between men and women (women had higher AL) and by diabetes were reported (Crews 2007).

INSERT TABLE 2 HERE

Statistical Analyses

First, we determine means and distribution for all biomarkers to determine quartile cut-points for each. Then, we examine Apo E and H, ACE, and ANP genotypes for significant associations with AL using t-tests and ANOVA. Next, we determine associations of age, sex, and education with AL. Following this, we use linear regression to determine independent associations of genotypes with AL controlling for age, sex, and education. We then determine differences in biomarkers between men and women and sex-specific biomarker distributions to compile sex-specific measures of AL. Next, we examine genotype associations with sex-specific AL. Finally, we determine associations of all other social factors with AL. Results are presented as differences between means, p-values and R^2-values for all three AL models. Given observed associations with genotypes, we also examine joint influences of apo E and ACE genotypes on AL using contingency analyses.

RESULTS

AL differs by genotype

Using sample-wide criteria for risk factor cutpoints (Table 3), those carrying either the apo 2,3 or ACE I-D genotype showed significantly lower AL (Table 4). For Model 1, AL averaged 1.92 of a possible 7 and ranged from 0-6. Models 2 and 3 averaged 1.50 (range 0-9 on an 11-point scale) and 3.33 on a 10-point scale (range 0-8).

AL differs by age, sex, and education

Using Model 1, men and women aged 55 or older had significantly lower AL ($\bar{x}$ = 1.98, 1.58; p = 0.095, 0.004) than those under 55 ($\bar{x}$ = 2.42, 2.58). This association persisted for Model 2 among men ($\bar{x}$ = 2.97; p = .081), but not women ($\bar{x}$ = 4.09 for younger women; $\bar{x}$ = 4.12 for older women). For the metabolic model, older women exhibited significantly higher AL ($\bar{x}$ = 3.37; p = .008) than younger women ($\bar{x}$ = 2.51), while older men had significantly lower AL ($\bar{x}$ = 2.47; p = .024) than younger ($\bar{x}$ = 3.27).

Given consistent differences in AL between men and women observed in these joint analyses, we examined sex differences in biomarkers (Table 4). Due to significant differences between men and women in 10 of the 17 biomarkers used to determine our three assessments of AL, we calculated AL models using sex-specific quartile cut-points (Table 4). American Samoan men have significantly higher diastolic blood pressure, w/h ratio, LDL- and HDL-cholesterol, triglycerides, fasting glucose, BMI, and skinfolds than women. Women show higher glycated hemoglobin and cholesterol than men. Additionally, women show significantly higher average AL than men (Figure 1). No significant associations of education with AL were observed in bivariate analyses (Figure 1).

INSERT TABLE 3 HERE

INSERT TABLE 4 HERE

INSERT TABLE 5 HERE

AL and genotypes controlling for age, sex, and education

AL differs significantly by education, age, sex and genotype among women. Model 1 AL is higher among younger women (Figure 1). Those with apolipoprotein E 3,4 and 3,3 genotypes show significantly higher AL than older women with the apo E 2,3 genotype ($\bar{x}$ = 2.49; p = 0.004 and $\bar{x}$ = 2.22; p = 0.098, respectively; Figure 2). Similarly, significantly lower AL ($\bar{x}$ = 1.63; p = 0.030) characterizes women reporting higher education and carrying an apo E 2,3 genotype than those without that combination ($\bar{x}$ = 5.50; lower education). For the body habitus AL model, no significant relationship with genotype, age, or education is observed among women. However, the metabolic model was related significantly to both age and AL. Older women had higher AL ($\bar{x}$ = 3.37; p = 0.008) than younger women ($\bar{x}$ = 2.51). Older women carrying the apo E 2,3 genotype have the lowest AL ($\bar{x}$ = 2.12; p = 0.029). Similarly, younger women with the ACE I-D genotype have the lowest AL (p = 0.044; Figure 3). Women with higher education and the apo E 2,3 or ACE I-D genotype also have lower AL than other women ($\bar{x}$ = 2.13; p = 0.004 and $\bar{x}$ = 2.50; p = 0.066, respectively). Using sex-specific AL, variable associations of apo E 3,2 and ACE I-D genotypes with age were confirmed. In all, we conducted 42 t-tests examining genotype associations with age, sex, or education and AL. Of these, 16 (38%) are significant (95% confidence interval; $t \geq 1.96$, $t \leq -1.96$).

INSERT FIGURE 1 HERE

INSERT FIGURE 2 HERE

Among men, AL related significantly to age and education (Figure 1). Men over age 55 had lower AL for both Models 1 ($\bar{x}$ = 1.98; p = 0.095) and 2 ($\bar{x}$ = 2.97; p = 0.081; Figure 1) than younger men ($\bar{x}$ = 2.42; 3.60). For Model 2, older men with the ACE I-D genotype had lower AL ($\bar{x}$ = 2.78; p = 0.110; Figure 3) than other men ($\bar{x}$ = 3.52; younger men). For AL Model 3, older men also display significantly lower AL than younger men ($\bar{x}$ = 2.47; p = 0.024; Figure 1). For the metabolic model, less educated men carrying the apo 2,3 genotype had lower AL than did others ($\bar{x}$ = 2.25; p = 0.071; Figure 2).

AL and additional social factors

Models 1 and 3 AL were significantly associated with the physical activity/drinking/smoking/blood pressure index (p=0.074 and p=0.016, respectively). Model 1 also correlated with a family history of death from stroke (p=0.071). Model 2 AL was associated with having a family member who died of a heart attack (p=0.050). Model 3, in addition to correlating with the index, was positively related to higher frequency of smoking and drinking (p=0.047 and 0.108) and having a family member who died of a stroke (p=0.047).

INSERT FIGURE 3 HERE

INSERT FIGURE 4 HERE

INSERT FIGURE 5 HERE

For both sexes and all models, associations of apolipoprotein H and ANP with AL were not significant. There was a borderline significant relationship observed among women with

more education and the ANP TB genotype (lower AL ($\bar{x}$ = 1.50) than the TT genotype and less education ($\bar{x}$ = 3.33 and 3.38; p = 0.106). Overall, AL was negatively associated with the ACE I-D and apo E 3,2 genotypes (Table 5).

DISCUSSION

Previous research shows AL predicts morbidity among Samoans as it does in other samples (McEwen & Stellar, 1993, McEwen 1998; Crews 2007; Evans et al., 2007; Hansson et al., 2008; Crews et al., 2012; Peters & McEwen, 2012). This study is among the first to assess relationships between AL and genotypes. Brody and colleagues (2013) examined relationships between AL, the DRD4 dopamine receptor gene, the 5-HTT polymorphic region, and family environment among African Americans aged 11-19. This earlier study conducted in rural Georgia (2004-2010) showed teens and young adults possessing DRD4 alleles with 7 or more repeats (the short allele at the 5-HTTLPR region) and experiencing unsupportive family environments had higher average AL than their counterparts. Smith and colleagues (2008) reported associations of AL with the ACE T allele, corticotropin-releasing hormone receptor 1 (CRHR1), and serotonin receptors (HTR3A and HTR4) among "Caucasian" adults residing in Sedgwick County, Kansas (1997-2000). Jointly, these results suggest neurohormone receptors are positively correlated with AL, as is at least one ACE polymorphism (rs4968591). Our report is the first to document relationships between AL and genotypes among any Polynesian or Asian sample. Examining genotypes at four loci, we observe two to significantly and consistently associate with AL, ACE and apo E. AL also is significantly associated with sex and education, but not age in our sample. Our hypothesis that AL would differ by genotypes in American Samoans was confirmed. Our second hypothesis, that associations with genotypes would be influenced by age, sex, and education, also was confirmed. Our expectation that higher AL

would occur among women than men was supported. However, older individuals did not show consistently higher AL than younger individuals. Our additional hypothesis that those with more education would exhibit lower AL also was not supported. We also hypothesized that AL would increase with "unhealthy" lifestyle habits, including lower physical activity and increased smoking, as well as with family history of having and/or dying from chronic diseases. These hypotheses were partially supported. For the selected genotypes, we predicted apo E 2,3, ACE I-D, ANP TB, and apo H 2,2 genotypes would be associated with lower AL. This was not so for ANP or apo H genotypes, which were little associated with AL.

Results reported here have some limitations. First, they are specific to American Samoa in the 1990s, but may be extrapolated to nearby related populations (Western Samoa, Samoans residing on the mainland and Hawaii) because of close similarities (see Crews and Lawson 2014). This sample consists of 273 individuals for whom we have detailed information, adequate for our main purpose, to determine associations of genotypes with AL in a Polynesian population, and concur with recent results from two other samples (Brody et al., 2013; Smith et al., 2008) in documenting that genotypic variation influences AL.

Methods of data measurement and collection were similar and highly accurate throughout the SSP (Baker et al., 1986). These techniques and the measures taken to mediate any discrepancies are detailed elsewhere (Bindon et al., 1997). One possible confounder is that in our sampling design we used a combination of directed and opportunistic sampling. Another possible confounder, as with previous studies in this population, is over time the SSP research came to be known as a health, blood pressure, and diabetes study. Some of our participants may have been motivated to participate by their poor health. Although we took direct steps to mediate this (see Bindon et al., 1997), it remains a possibility. However, this does not alter results reported here as

we are not examining health, but rather associations of phenotypes with genotypes and the influences of age, sex, and education on observed associations.

Associations of AL with apo E and ACE genotypes remain after controlling for age, sex, and education (Figures 2-5). Lack of associations between AL and apolipoprotein H or ANP may arise from many factors, including a true lack of association. For another, the particular constructs used to assess AL may not address effects of these loci, or specific physiological traits of American Samoans may limit such association. Additionally, these loci may be poorly associated with AL as a composite measure of physiological dysfunction, although being significantly associated with several of its specific components (e.g., blood pressure or cholesterol). Several borderline relationships of ANP with AL suggest such associations, although not strong in this sample, may exist in other populations.

Both biological sex and education associate significantly with AL, although age does not. It is not likely that the latter reflects a limited age range in our sample, which is over 50 years (ages 35-87). Both the body habitus and metabolic AL assays are lower among women who report higher education, but Model 1 AL is not (Figure 1). Men who did not complete high school and also carry the apo E 2,3 genotype have significantly lower AL than others. One interpretation is that among American Samoan women education contributes to a higher AL, while among men, the effect of education on AL partly depends on apo E genotype.

Previously, we showed that American Samoan women experience higher overall AL than men (Crews 2007). This finding is confirmed and differs from other samples wherein men tend to have higher reported AL (McEwen 2000; Schnorpfeil et al., 2003; Duru et al., 2012). However, in a Japanese sample AL also was higher in women (Crews et al., 2012). Contrary to

our other hypotheses, older individuals do not consistently show higher AL (Figure 1). Rather, women under age 55 experience higher AL (Model 1). However, using Model 3 (metabolic), women aged 55 and over have higher AL. Age may influence AL among women, but its specific influence is indeterminate as any association is dependent upon how AL is assessed. Measures of AL are not standardized and thus different models of AL yield different results. Here, we use three models to account for some of this difference. Conversely, regardless of AL model, lower AL characterizes American Samoan older men (Figure 1), suggesting a negative association. This may reflect a true difference between younger and older men, or men with higher AL may not have survived sufficiently long to be in our sample. Previous results suggest family and social status, along with region of residence and lifestyle, influence risk factors and mortality among American Samoans (see Baker et al., 1986; Crews 2007). Such factors likely modulate adult AL as well. Early life social and environmental factors also likely affect activity levels, access to nutritious foods, and morbidity/mortality (see Baker et al., 1986; Crews 1989, 2007). Although these may influence AL, the consistent association of genotypes at two unrelated loci provides clear evidence that AL is mediated by genotype and genotype by sex and age interactions.

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Table 1. Components of allostatic load (following Seeman et al, 1997; McEwen et al, 1999)

Primary Stress Responses
Glucocorticoids (cortisol)
Catecholamines (adrenaline and noradrenaline)
DHEA-S
Secondary Stress Responses
Glucose handling (glycated hemoglobin)
Cardiovascular reactivity (systolic and diastolic blood pressure)
Lipid metabolism (Serum HDL- and total-cholesterol)
Energy Storage (waist/hip ratio)

Table 2. Components of three assessments of allostatic load (AL): Model 1: secondary mediators of stress and physiological disregulation (after Seeman et al 1997) plus fasting insulin, Model 2: additional measures of body habitus, and Model 3: addition of measures of metabolism to Model 1

Model 1	Model 2	Model 3
SBP	SBP	SBP
DBP	DBP	DBP
Chol	Chol	Chol
w/h	w/h	w/h
GlyH	GlyH	GlyH
HDL	HDL	HDL
FSI	FSI	FSI
	RFPI	LDL
	TrSf	Trig
	SbSf	FSG
	BMI	

Notes: SBP/DBP – systolic/diastolic blood pressure; w/h – waist/hip ratio; GlyH – glycated hemoglobin; Chol – total cholesterol; HDL/LDL – high/low-density lipoprotein cholesterol; Trig – triglycerides; FSG/FSI – fasting serum glucose/insulin; BMI – body mass index; RFPI – relative fat pattern index; TrSf/SbSf – triceps/subscapular skinfolds

Table 3. Averages, standard deviations, ranges, p-values, and quartile cut-points for men and women of biomarkers available for computing allostatic load (AL) composites among 273 American Samoans (123 men, 150 women).

				Quartile cut-point*
	Mean	(s.d.)	Range	All
Age	55.5	(9.8)	35 – 88	62.6
SBP	141.2	(24.8)	88 – 236	155.0
DBP	085.1	(16.1)	10 – 140	93.0
w/h	91.0	(7.1)	72 – 112	96.0
GlyH	10.0	(6.0)	5 – 90.3	10.6
Chol	193.1	(37.8)	87 – 325	218.0
HDL	28.4	(8.4)	12 – 70	22.7
LDL	138.8	(63.0)	114 – 999	159.0
Trig	148.8	(37.8)	87 – 325	175.0
FSG	158.0	(76)	34 – 492	188.0
FSI	167.0	(206)	14 – 2495	189.0
BMI	28.8	(5.8)	14 – 51	32.0
RFPI	0.5	(0.04)	0.31– 0.72	0.50
TrSf	35.9	(16)	6 – 60	49.2
SbSf	50.6	(13)	8 – 69	60.0

Notes: SBP/DBP – systolic/diastolic blood pressure; w/h – waist/hip ratio; GlyH – glycated hemoglobin; Chol – total cholesterol; HDL/LDL – high/low-density lipoprotein cholesterol; Trig – triglycerides; FSG/FSI – fasting serum glucose/insulin; BMI – body mass index; RFPI – relative fat pattern index; TrSf/SbSf – triceps/subscapular skinfolds. *For HDL, the cutpoint is the upper-bound value of the first quartile.

Table 4. Differences in allostatic load (AL) by genotypes at the apolipoprotein (apo) E and angiotensin converting enzyme (ACE) loci in a sample of American Samoan (N=273) aged 35-87.

	Apo E				ACE		
	3,3	2,3	3,4	p*	I-I	I-D	p*
AL 1	1.84	1.53	2.26	.0251	2.09	1.64	.0306
AL 2	3.29	3.13	3.67	.3471	3.53	3.05	.1226
AL 3	2.59	1.97	3.20	.0057	2.91	2.29	.0320

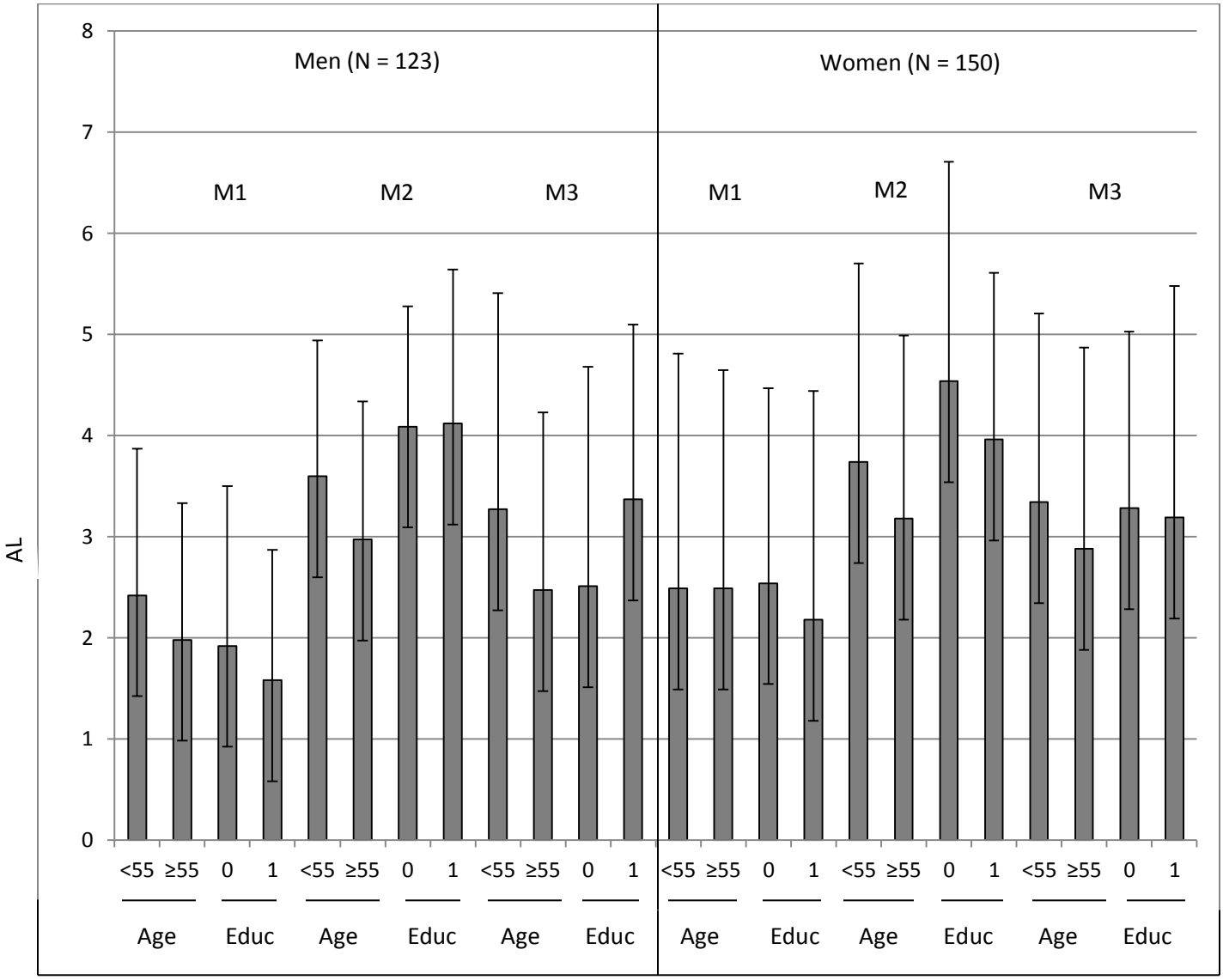
p-values for tests comparing average AL by apo E ANOVA, ACE t-test. Four out of six tests are significant; probability with Bonferroni Correction: chance of finding one or more significant differences in six tests = 26.5%.

Table 5. Differences in averages of allostatic load (AL) in American Samoan men (N = 123) and women (N = 150)

					Risk quartile cutpoints	
	Men	Women	Difference	p	Men	Women
SBP	142.1	140.5	1.6	0.593	154.0	156.0
DBP	86.5	82.4	4.1	0.028	96.0	92.0
w/h	95.0	87.8	7.2	0.000	99.0	91.5
GlyH	9.61	10.16	-55.8	0.455	10.20	10.60
Chol	191.5	195.0	-3.5	0.451	212.0	222.0
HDL*	26.9	29.7	-2.8	0.066	30.9	33.8
LDL	130.3	140.6	-10.3	0.017	154.0	163.0
Trig	177.5	123.2	54.3	0.000	200.0	137.0
FSG	166.5	148.5	18.0	0.078	211.0	179.0
FSI	169.1	168.2	0.9	0.972	169.0	214.0
BMI	28.1	29.5	-1.4	0.048	31.2	33.1
RFPI	0.50	0.50	0.00	0.310	0.53	0.50
TrSf	24.9	44.8	-19.9	0.000	32.2	55.3
SbSf	44.3	55.8	-11.5	0.000	57.0	60.0

Notes: SBP/DBP – systolic/diastolic blood pressure; w/h – waist/hip ratio; GlyH – glycated hemoglobin; Chol – total cholesterol; HDL/LDL – high/low-density lipoprotein cholesterol; Trig – triglycerides; FSG/FSI – fasting serum glucose/insulin; BMI – body mass index; RFPI – relative fat pattern index; TrSf/SbSf/SSf/McSf – triceps/subscapular/suprailiac/medial calf skinfolds. Difference = mean AL for men – mean AL for women. * For HDL, the cutpoint is the upper-bound value of the first quartile.

Figure 1. Average allostatic load (AL) by age, sex, and education among American Samoan adults aged 35-87 (N = 273)



Notes: M1 = Model 1 (secondary mediators); M2 = Model 2 (body habitus); M3 = Model 3 (metabolic). Educ = education; 0 = less than high school graduate; 1 = high school graduate or higher. Bars represent 95% confidence intervals.

Figure 2. Average allostatic load (AL) by age, sex, and apolipoprotein (apo) E genotype among 273 American Samoans (M1: secondary mediators, M2: body habitus, M3: metabolic)

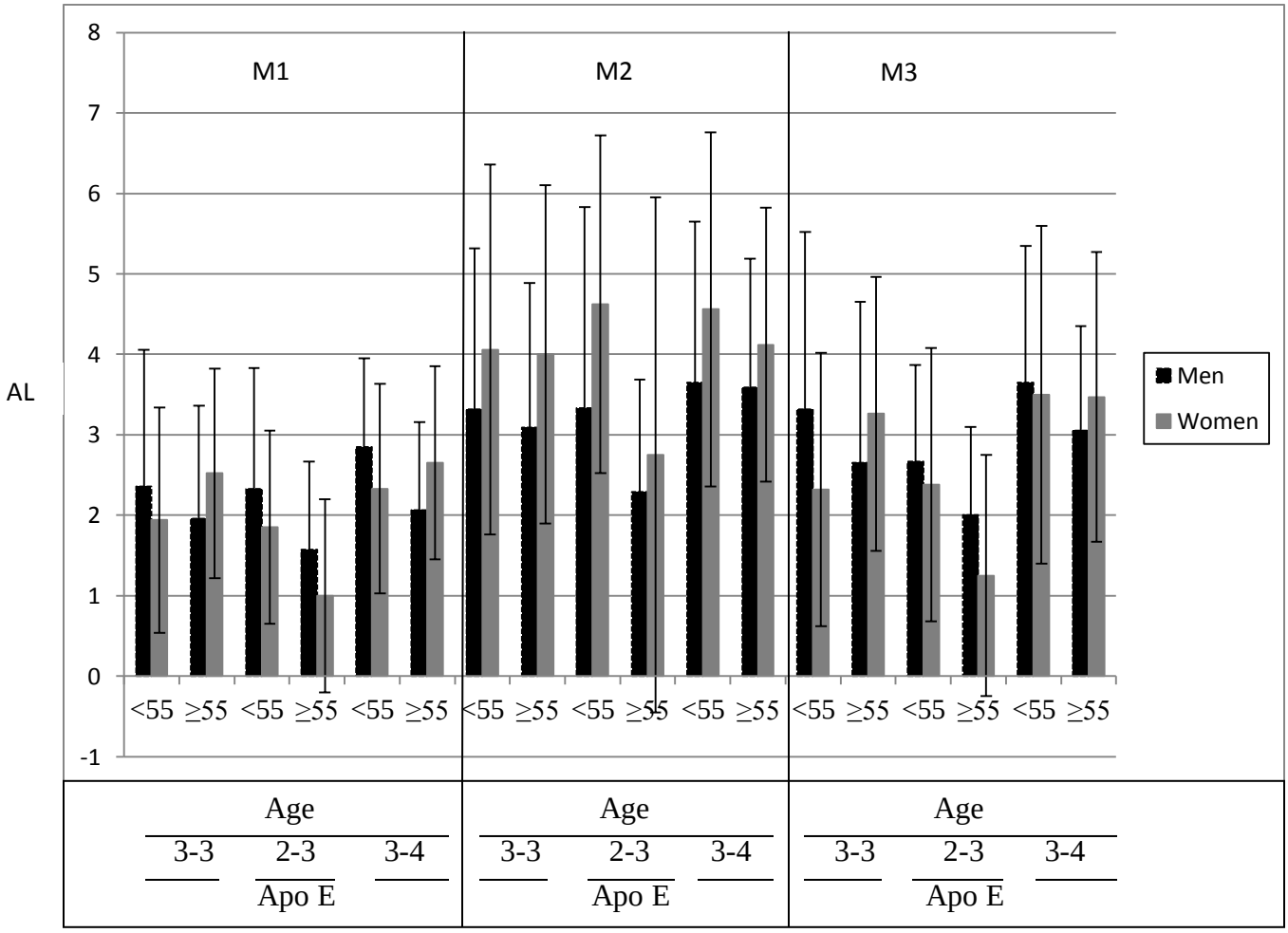


Figure 3. Average allostatic load (AL) by age, sex, and angiotensin converting enzyme (ACE) genotype among 273 American Samoans (M1: secondary mediators, M2: body habitus, M3: metabolic)

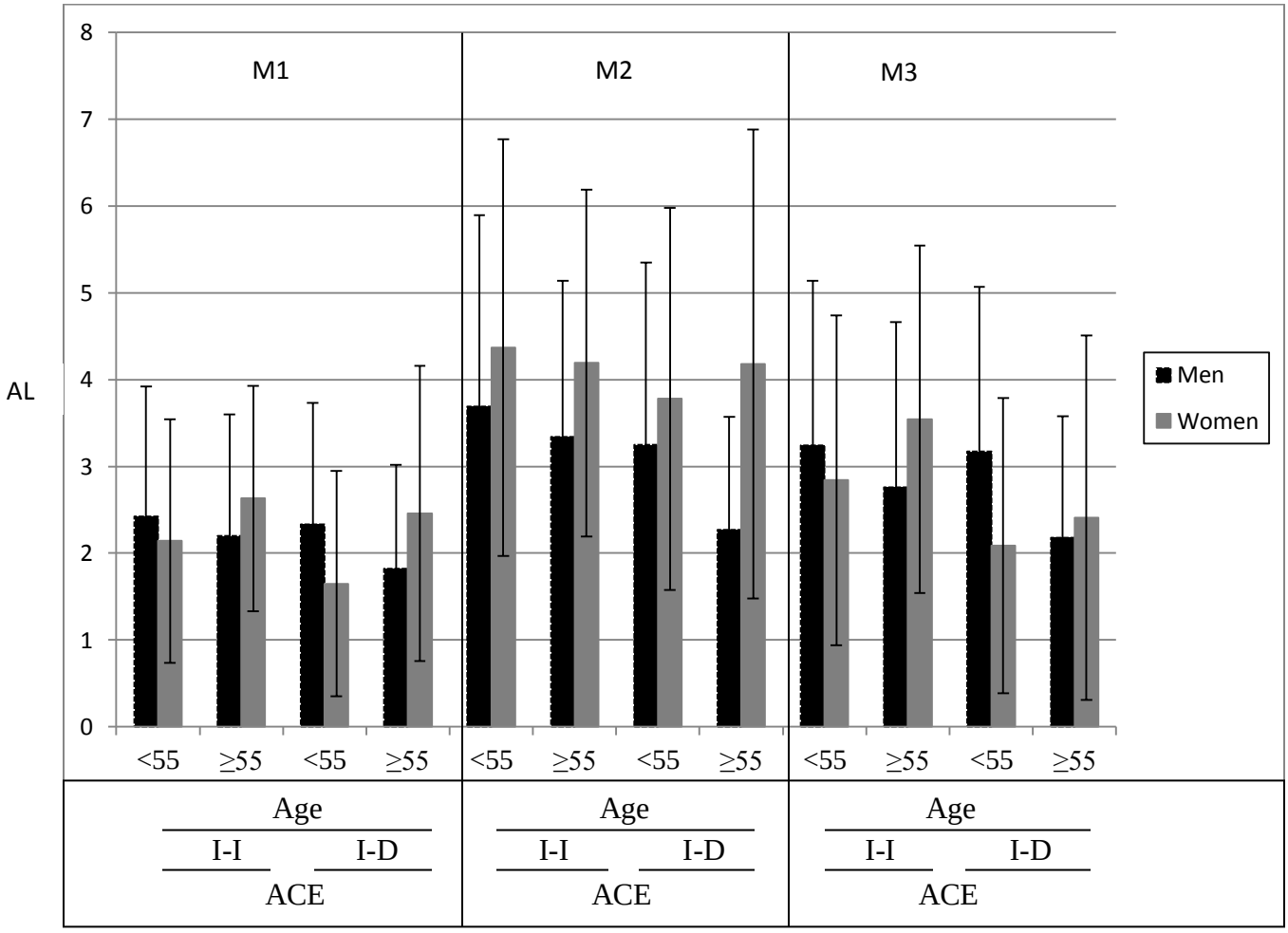
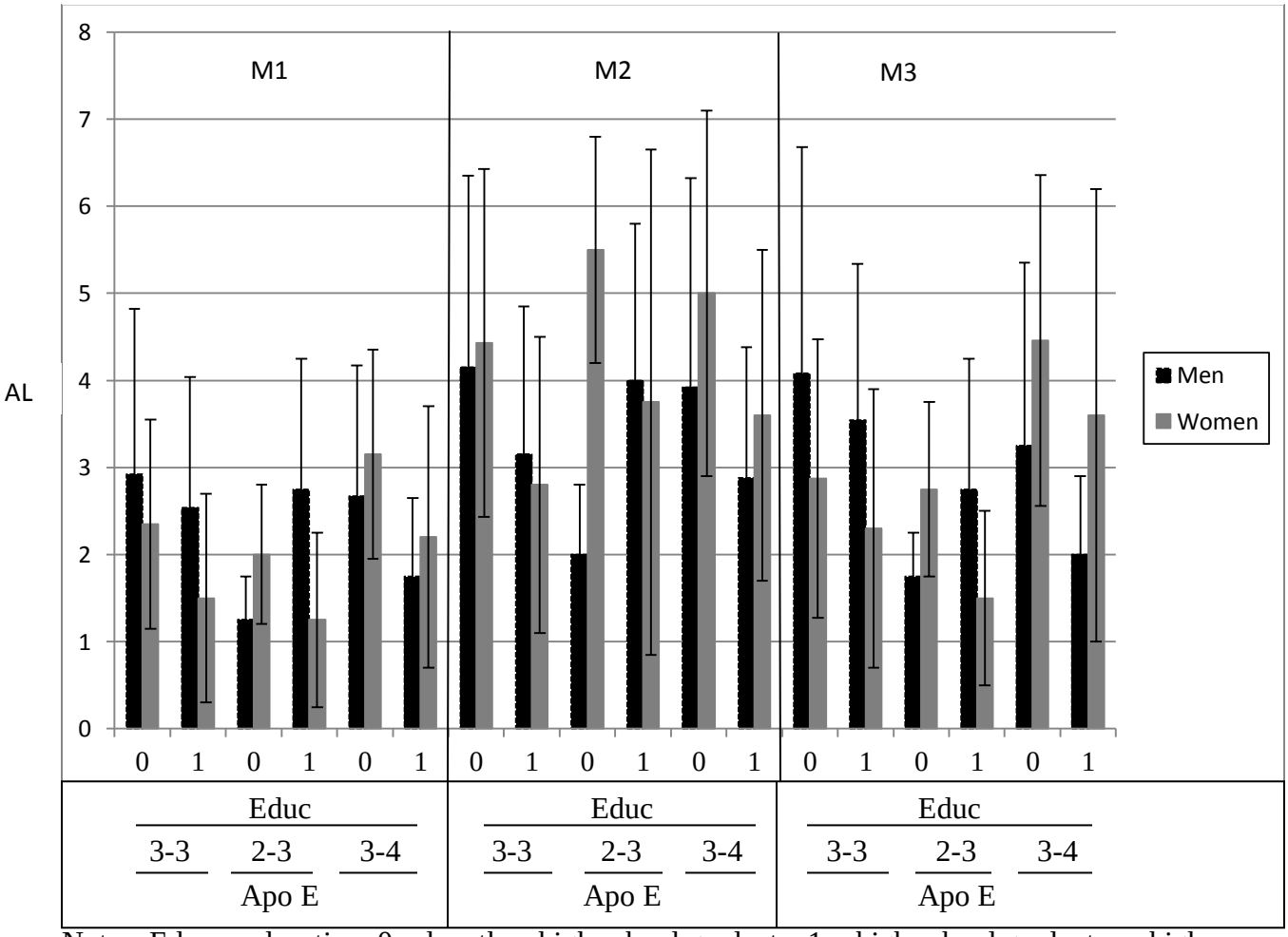
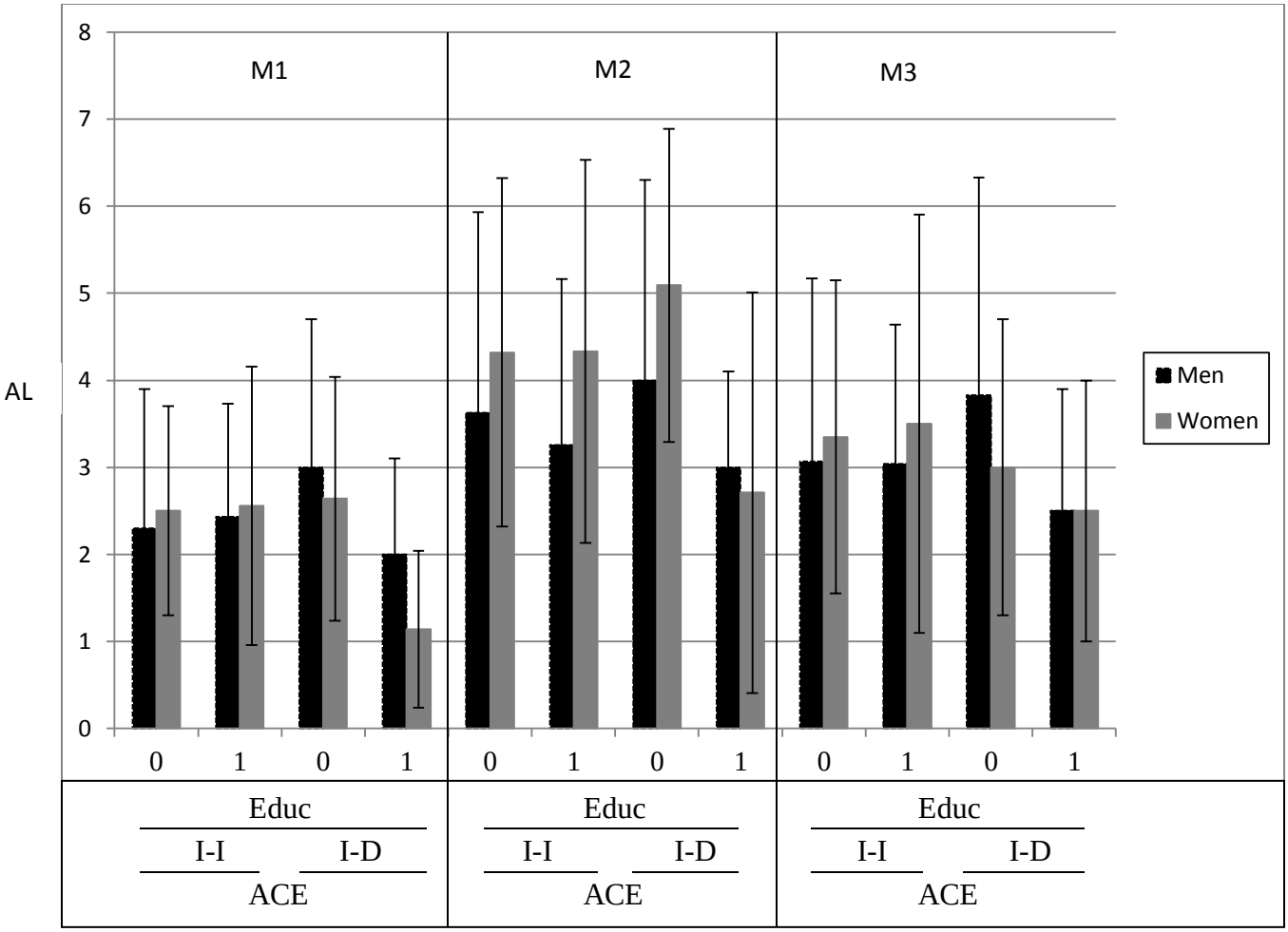


Figure 4. Average allostatic load (AL) by education, sex, and apolipoprotein (apo) E genotype among 273 American Samoans (M1: secondary mediators, M2: body habitus, M3: metabolic)



Notes: Educ = education; 0 = less than high school graduate; 1 = high school graduate or higher.

Figure 5. Average allostatic load (AL) by education, sex, and angiotensin converting enzyme (ACE) genotype among 273 American Samoans (M1: secondary mediators, M2: body habitus, M3: metabolic)



*Educ = education; 0 = less than high school graduate; 1 = high school graduate or higher

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